THE RELATIONS BETWEEN THE INTERNAL FLUID OF MARINE INVERTEBRATES AND THE WATER OF THE ENVIRONMENT, WITH SPECIAL REFERENCE TO AUSTRALIAN CRUSTACEA.

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(Five Text-figures.)

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During the last forty years considerable interest has been shown in the osmotic and chemical relations between the body fluids of aquatic animals and the surrounding water. To some extent the results of experiments, made to determine the degree of physiological dependence between the two media, have been conflicting and, in the endeavour to discover the forces regulating the internal fluids, there has been need for fundamental changes in our conception of the phenomena as the number of investigations has increased. Naturally much attention has centred round the question of the permeability of the surfaces separating the two media.

The study of this work was introduced to me by Professor Dakin of the University of Sydney, and the experiments described in this paper were made at his instigation and in continuation of work conducted by him (Dakin, 1912). Their purpose was the relation of the behaviour of certain Australian aquatic invertebrates in sea-water and in fresh water to the behaviour of those already examined in other countries. Brief reference will, therefore, be made to the results of certain other investigators.

Condition of the Blood of Marine Invertebrates when immersed in ordinary Sea-Water.

Before examining the osmotic conditions of the blood of animals immersed in experimental solutions of sea-water, it is necessary to be certain of the osmotic pressure conditions existing under normal circumstances. It has frequently been stated that when crustaceans and other invertebrates are living in ordinary sea-water the blood has exactly the same osmotic pressure as the surrounding water.

In recent years, however, several workers have shown that this is an inadequate expression of the conditions actually prevailing. Bottazzi (1897) was satisfied with finding that the mean (−2·29°) of the freezing points obtained for the blood of all the invertebrates examined, was equal to the average freezing point of the water in the same locality. For a more accurate result it would appear necessary to measure the freezing point of the water from which the animals have actually been taken and for every case separately. Retention in a laboratory for some time is frequently the only method of doing this with certainty.

Most of the early workers noticed slight differences between the $\triangle$ of the body fluids and the $\triangle$ of the sea-water. But the differences were only of the order 0·03° or thereabouts. More recently M. Duval investigated a number of species of marine invertebrates in their natural state and compared the freezing point of their blood with that of the external water. He found that the difference between the two freezing points never exceeded 0·02°, which he regards as merely of the order of experimental error, and consequently speaks of the media as "exactement isotonique". It is perhaps natural that this small difference should have been disregarded by the earlier investigators, and come only to be considered as significant when it was found that a larger difference occurred in some species.

Schlieper (1929) found that in the crab *Carcinus maenas* the concentration of the blood was never equal to that of the external medium. The difference, though still slight, was too great to be due to experimental error. It varied from 0·04° to 0·09°, the blood having always the higher concentration.

Monti, quoted by Schlieper, finds a greater difference between the △ of blood and of the external water in the case of *Carcinus maenus*. He gives the latter as 1·96°–1·99°, the former as 2·17°. Again in the Mollusca, Monti finds the internal fluid to be hypertonic to the sea-water. For example, in water of △ 2·11°–2·14°, he obtained △ 2·23, 2·26 and 2·16 for the internal fluid of an oyster, mussel and octopus respectively.

(It is interesting to note that Duval and Prenant have shown the same thing for ascidians. (Blood △ = 2·08°; sea-water △ = 1·98°.) Although these animals belong to the phylum Chordata, their behaviour would seem better comparable to that of invertebrates than that of the higher chordates.)

On the side of hypotonicity of the blood to the surrounding medium, Baumberger and Olmstedt have discovered a very striking example in *Pachygrapsus crassipes* where, under normal conditions, the △ for the blood is 1·327° for an external △ of 1·975°.

Thus the older view that there is *complete* isotonicity between the internal and external media of marine invertebrates has had to be modified. My examination of a number of species of Australian crabs furnishes further proof that complete isotonicity is by no means of universal occurrence among the invertebrates.

The crab *Heloecius cordiformis* has proved a very suitable Australian invertebrate for this type of investigation.* This species is common near the coast of New South Wales and occurs in very great numbers on mangrove flats, which are uncovered at low tide. The depression of the freezing point was taken as a measure of the osmotic pressure and given the usual designation of △. It was determined by a Beckmann apparatus. The crabs are so small that a single specimen does not furnish enough blood for a freezing point determination, the blood from three, four, or five being necessary for each determination. The blood was obtained by cutting one of the chelae. During all the experiments described in this paper the crabs were kept in water which was either constantly stirred or else aerated by the bubbling through it of air under pressure.

It was found that when *Heloecius cordiformis* is immersed in ocean sea-water the blood is markedly hypotonic. Specimens were kept for about a week in sea-water brought from their own locality. (This was far more than the time required for the cessation of any change in the blood which might occur if the water was somewhat different from that in which they were immersed prior to capture.) It was found that the depression of the freezing point for the blood was about 0·25° less than that of the water. Below is a table giving some of these results.

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* *Heloecius cordiformis* was selected by Professor Dakin and was used in the experiments reported on by Dakin and Edmonds (1931).

TABLE 1.

Time of Immersion.	△ of Medium. (° C.)	△ of Blood. (° C.)	Difference. (° C.)
6 days	2·21	1·92	0·29
7 days	2·18	1·96	0·22
8 days	2·16	1·95	0·21
8 days	2·16	1·89	0·27
6 days	2·23	1·88	0·35
16½ hours	2·17	1·99	0·18
16½ hours	2·17	1·95	0·22

It is strange that the inequality in this case should be on the side of hypotonicity of the blood, whereas it was on the side of hypertonicity in *Carcinus maenus*, the crab which has been so much used for European experiments, and which is found under similar conditions. Anything but a small degree of hypotonicity seems to have been recorded previously only for *Pachygrapsus*.

But the hypotonicity is not confined to the one species of Australian crabs. The rock crab, *Leptograpsus variegatus*, which occurs very abundantly on the coast around Sydney, was also examined. Specimens of the crab were taken straight from the rocks below the Biological Station at the mouth of the harbour, and there put into running or aerated sea-water. The depression of the freezing point of the blood was found to be approximately 0·16° less than that of the water.

TABLE 2.

Blood (0° C.).	Sea-Water (0° C.).
1·99	2·13
1·95	2·13
1·97	2·15 (approx.)

For some reasons it was more interesting to discover this in *Leptograpsus* than in *Heloecius*. As the latter occurs on river flats, with a distribution from the sea to the places where the water contains very little salt, it might be expected that the position of equality would be at some place (and concentration) between the two extremes. But *Leptograpsus* is found very commonly all along the actual coast and so normally lives in ocean sea-water.

The third crab examined for its △ in normal sea-water was *Sesarma erythrodactyla*, which is found on mangrove flats and river banks at the same places as *Heloecius*. This crab is even smaller than *Heloecius* and it is therefore necessary to use a number of specimens for each determination. It was more difficult to obtain precise results for this crab, because it exhibits a greater variability of internal concentration under the same external conditions than do the species previously considered. However, it would seem that in this crab, too, the concentration of the blood is below that of the sea-water in which it normally lives. A number of specimens collected from a mud flat near the sea were kept for twenty days in water with a freezing point of −2°. Two determinations of the freezing point of the blood at the end of this period gave −1·85° and −1·91°. One would expect (in the light of facts which will be set forth later) that in fully concentrated sea-water ($\triangle = 2{\cdot}15°$ approximately) the difference of the internal from the external medium might be slightly greater.

Certain fresh-water crabs (at present undescribed) from the Hawkesbury River also provide data interesting in this connection. When this species is introduced into sea-water its blood increases in concentration, but does not, even after several weeks' immersion, reach the point of isotonicity with the surrounding water. The △ of the blood of such specimens was 1·91°, with an external △ of about 2·13°, giving a difference of 0·22°, which is similar to the difference in the case of *Heloecius*.

The Osmotic Conditions of the Blood of Marine Invertebrates in dilute Sea-Water, and in Sea-Water of increased salinity.

From this preliminary discussion of the relations between the internal and external fluids in normal sea-water, we can now consider these relations when the conditions are varied by the dilution or concentration of the sea-water. In the natural state, certain marine species are found distributed over a wide variety of concentrations of sea-water, perhaps with little movement from place to place within this range. Such are a number of species which inhabit the salt and brackish portions of rivers. It is chiefly with Crustacea of this type that my own investigations have been carried out.

There are others which are found only where the sea-water is fully concentrated, but which can withstand certain abnormal salinities, as has been shown by experiment.

It was originally held that the internal fluid of marine invertebrates was "the plaything of the conditions of their environment". Early experiments confirmed this view and suggested that complete isotonicity was attained after alteration in the salinity of the external medium. Later, however, it was found that whilst this held to a certain extent for echinoderms, a number of molluscs and some worms, in other groups the internal medium was not so dependent upon the external and, in fact, isotonicity was far from attained after alterations in the salinity of the latter.

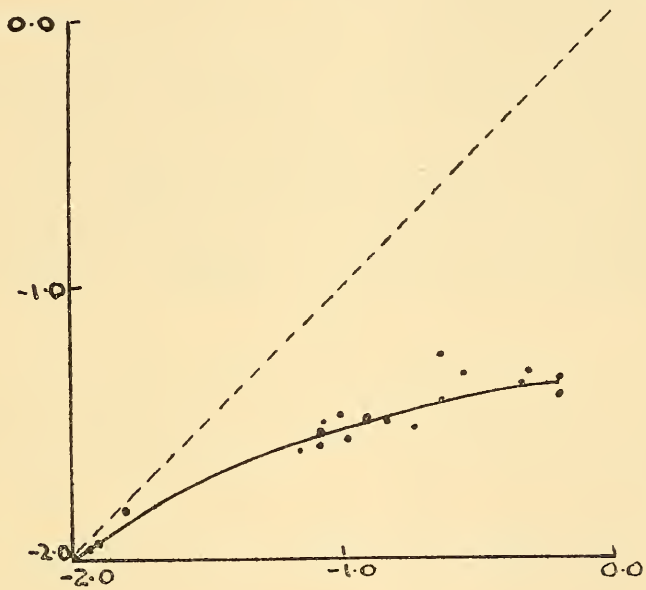
A number of workers have now indicated the diversity of the reactions of marine invertebrates in this respect. Duval, for example, found that even in the typical marine crabs, *Platycarcinus pagurus*, *Maja squinado*, and *Portunus puber*, the salinity of the blood remained higher than that of the external medium when the salinity of the latter was reduced to a point where its △ was 1·4°C.

Much of our recent information has been obtained by extensive experiments using the crab *Carcinus maenas*, which occurs on the European coast and in brackish water at the mouths of rivers. It is worth setting forth these experiments at some length, firstly because *Carcinus* is so admirably suited for this type of experiment, being able to withstand great changes in salinity, and secondly because this crab makes an interesting comparison with the Australian crab, *Heloecius cordiformis*. Both these crabs occur naturally in sea-water of a wide range of salinities, and the concentration of their blood is similarly affected by a low external salinity.

Frédéricq (1901) took specimens of *Carcinus* and put them into dilute solutions. He found that these crabs were slow in adapting themselves to the new medium. From his results it was not clear whether the outer and inner media would have become equal in molecular concentration if the time of the experiment had been extended, or whether the blood of the crabs would have remained permanently hypertonic to the external water. The latter case appeared the more likely in the light of Frédéricq's previous discovery of the excess of calcium chloride in the blood above that in the brackish water which members of the species inhabit in nature.

Subsequently, experiments by Duval and by Schlieper proved conclusively that this difference in salinity was not the result of a slow establishment of equilibrium, but always occurred for anything but very slight dilutions. (Duval found that it required not more than twenty-six hours to reach a state where the △ of the blood remained constant.)

Schlieper (1930) obtained the same results as Duval for *Carcinus maenas* placed in dilute solutions and his results are summed up in Figure 1, where the dotted line indicates the curve for the freezing points of the external medium and the unbroken line that for the blood.



Text-fig. 1.

The results for *Carcinus maenas* taken together provide a very convincing proof that the "law of poikilosmoticity" is by no means universally applicable among marine invertebrates.

That this is not an exceptional case has been shown by the examination of several species of Australian crabs. In none of these species is there isotonicity between the external and internal media when immersed in a diluted sea-water (except for the special case of a solution just below the concentration of ocean water. This will be explained later).

I shall now give an account of the behaviour of certain of these Australian crabs in dilute solutions, both under conditions of nature and in laboratory experiments.

The dilute solutions were made by mixing fresh tap-water with sea-water. For convenience the different dilutions will be spoken of as percentages. Thus a 100% solution means one composed entirely of normal sea-water, a 75% solution is one made from 75% of sea-water, 25% of fresh, and so on. As before, the crabs were kept, several at a time, in glass jars in which the water either was stirred or had air constantly bubbling through it. The crabs were put straight into the new medium without any sojourn in an intermediate concentration. In all cases the depression (△) of the freezing point below 0° C. was taken as the measure of osmotic pressure.

It will be best to commence with a description of the phenomena for *Heloecius cordiformis*, as it is with this crab that the investigations have been most thorough and extended. They were commenced by Professor Dakin in 1929 and first reported on by Dakin and Edmonds in 1931. Since then the experiments have been extended and carried out on a large scale to eliminate the risk of chance variations giving an erroneous picture of the real facts. *Heloecius cordiformis* is particularly suitable for such experiments, for it will live well when suddenly transferred from normal sea-water into almost any mixture of salt and fresh water, though it dies quickly in absolutely fresh water. Very few (out of large numbers of specimens) have been kept in fresh water for as long as two days. It is rather amazing to note the difference in survival, which can be brought about by the addition of a very small amount of sea-water to the fresh. Thus in one experiment of 30 hours' duration, where the solutions were 0%, 2%, 4% and upwards, the crabs in the fresh water were dead, but the others survived. Still more striking are the differences recorded in Table 3, where the amount of sea-water seems almost inappreciable. (See also Dakin, 1908.)

TABLE 3.

Heloecius cordiformis *after immersion of 41 hours.*

Fresh Water.	0·5% Sea-Water. 99·5% Fresh Water.	1% Sea-Water. 99% Fresh Water.	1·5% Sea-Water. 98·5% Fresh Water.	2% Sea-Water. 98% Fresh Water.
Dead: 7 Alive: 1	Dead: 1 Dying: 2 Alive: 5	Dead: 3 Dying: 2 Alive: 3	Alive: 8	Alive: 8

Before discussing the question of isotonicity or anisotonicity between the two media for this crab, it is necessary to have some idea of the time required for the cessation of the internal changes which are induced by the external change, i.e., for the attainment of a new equilibrium. Table 4, for a 25% solution, indicates that the internal change takes place at first quickly, then slows down, and may be regarded as practically complete after about twelve hours. As will be shown later, differences up to a tenth of a degree occur as a result of the variation between different crabs when in the same solution for the same length of time.

TABLE 4.

Heloecius cordiformis *immersed in water 25% sea-water, 75% fresh water.*

Time of Immersion.	△ of blood. (° C.)
2 hours	1·96
7¾ hours	1·78
10½ hours	1·75
12 hours	1·75
62 hours	1·70

In the experiments for determining the effect of dilute solutions, however, to make accuracy more certain, the crabs have been left for a longer period, usually thirty to forty hours. The time of immersion is indicated wherever tables are given. Naturally it would be expected that the farther from normal the external salinity is, the longer will be the time before completion of the change. This is borne out by a comparison of the results of Table 4 with those for fresh water. Table 5 and Figure 2 indicate that when the crabs are immersed in fresh water the point of constant salinity for the blood is not reached in twenty-four hours, and it is impossible to judge whether it is reached even in forty-four hours, as death prevents the time from being extended.

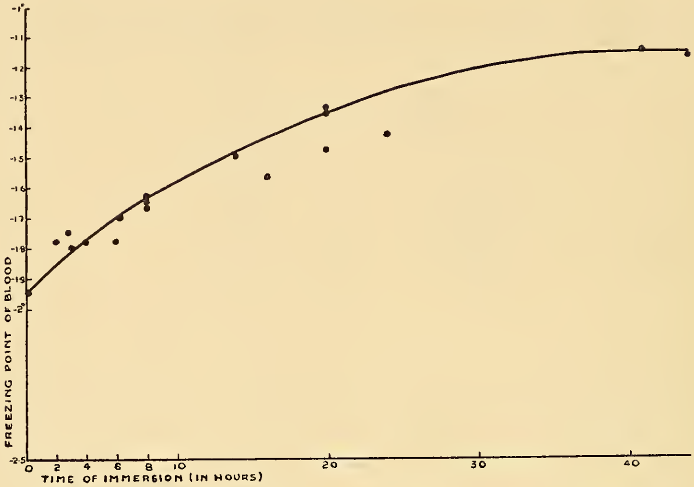
TABLE 5.

Heloecius cordiformis *immersed in fresh water.*

Time of Immersion. (In hours.)	△ of Blood. (° C.)
0	1·95
2	1·78
3	1·80
4	1·78
6	1·78
8	1·65
14	1·50
16	1·57
20	1·36
24	1·43 (approx.)
41	1·15
44	1·17

In the experiments which are now to be considered, for solutions other than fresh water and for very low salinities, the changes may be regarded as complete at the time when the blood was taken from the crabs.

The more the water was diluted, the more also did the blood of the crabs become diluted, the concentration of the blood decreasing at much the slower rate, until, for very low external concentrations (20% solution and less), further dilution seemed to cause little, if any, additional change in the concentration of the blood.



Text-fig. 2.—*Heloecius cordiformis* immersed in fresh water.

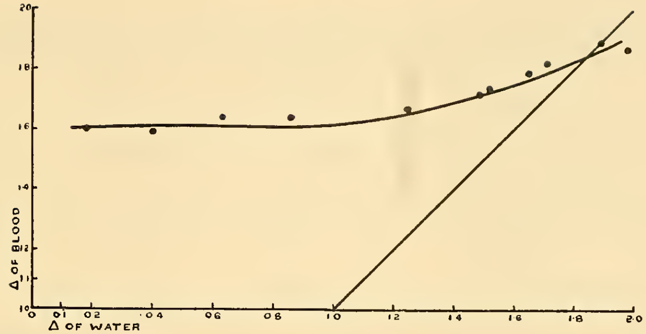
As a result of this slower rate of decrease in the internal concentration, the two media are of course anisotonic in dilute solutions, the inner one becoming more and more hypertonic as the dilution is increased. This is very evident from Table 6 and Figure 3.

TABLE 6.
Heloecius cordiformis *immersed for 34 hours in dilute solutions.*

Percentage of Sea-Water in the Solution.	△ of Solution. (° C.)	△ of Blood. (° C.)
100	2·19	2·19*
90	1·98	1·87
80	1·71	1·82
70	1·52	1·74
60	1·25	1·67
40	0·86	1·64
30	0·63	1·64
20	0·40	1·59
40 hrs. 10	0·18	1·60

* It is most unusual for the internal and external △ to be equal at this salinity. The corresponding point has therefore been omitted from the graph.

In order to make quite certain that this hypertonicity was not due to a very slow rate of change in the osmotic pressure of the blood while the animals were becoming accommodated to the new salinity of the environment, a number of crabs were kept in the diluted sea-water for longer periods than those already indicated. A series of dilutions in which the time of immersion was six to eight days still showed the discrepancy between the internal and external △. The former gave a △ as large as 1·62 in a solution containing only 40% sea-water.



Text-fig. 3.—*Heloecius cordiformis* immersed for 34 hours in dilute solutions of sea-water. (The diagonal represents equality of △ in the two media.)

Moreover, on two occasions, enough crabs for a freezing point determination were kept in a diluted sea-water for several months. At the end of this time the blood was still found to be markedly hypertonic to the water. Below are the results of one such case.

Heloecius cordiformis.

	△ of Water. (° C.)	△ of Blood. (° C.)
Two months' immersion	0·72	1·38

It has been already shown that, under normal circumstances of 100% sea-water, the blood has a decidedly lower osmotic pressure than the surrounding water. As a consequence of this, when very slight dilution of normal water takes place, the slower rate of internal change at first brings the osmotic pressures of the two media closer together, until for one point (roughly between 80% and 90%) the two become identical.

In a previous paper (Dakin and Edmonds, 1931) we have stated that for *Heloecius cordiformis* the blood is isotonic with the sea-water within certain limits and that this crab is "another example of a marine crustacean which is . . . . poikilosmotic in the sea . . . . but is homoiosmotic in diluted sea-water". We have since found that this statement is not quite accurate. The inaccuracy, however, does not invalidate the conclusions drawn in the paper in question. It is more important in the present connection.

Further, more precise investigation has revealed, for *Heloecius cordiformis*, that the occurrence of isotonicity is so restricted that it can hardly be spoken of as a "range" at all. In Table 7 the points where the internal and external △ are identical in different experiments are very close together (the exceptional case must have some other explanation).

Finally, to confirm these experimental results, a series of crabs was collected along the Hawkesbury River, N.S.W., beginning at Wiseman's Ferry (which is the part of the river where the mangrove flats frequented by *Heloecius cordiformis* occur farthest from the sea) and continuing down the river to the mouth. The figures for the △ of the blood obtained for these crabs accord well with the experimental ones, and show that it was right to conclude from the latter that the

TABLE 7.
Heloecius cordiformis *immersed in Sea-Water solutions which permitted of isotonicity.*

Duration of Immersion.	△ of Water. (° C.)	△ of Blood. (° C.)
44 hours	1·89	1·89
34 hours	2·19	2·19 (exceptional)
8 days	1·77	1·75
8 days	1·77	1·75
Some days	1·85	1·83

blood is hypertonic to dilute external media and that the hypertonicity becomes very great when the external △ is small. The results obtained along the river are given below (Table 8) and compared with the experimental ones. The first column of figures gives the △ for the water at both high and low tides, the last column gives the △ of the blood under experimental conditions, where the external medium was of about the same salinity as the water of the part of the river under consideration. The comparison cannot be very precise, because the △ of the river water can be only approximately that of the water in which the crabs were actually immersed at or before the time of capture. The similarity between the two columns is, however, marked enough to indicate that the laboratory results are not artificially induced by the unusual conditions, but that they are valid as a record of the effect of change in external concentration only.

TABLE 8.
Heloecius cordiformis *under Natural Conditions—Comparison of blood △ with that from crabs of same species in Experimental Solutions of similar Concentration.*

Place.	△ of Water. (° C.)	△ of Blood. (° C.)	△ of Blood in Experimental Solutions of similar salt-content. (° C.)
Wiseman's Ferry	0·38–0·58	1·43	1·59
,, ,, ...	,, ,,	1·47	
,, ,, ...	,, ,,	1·39	
Laughtendale	0·62–0·84	1·47	1·38
,, ...	,, ,,	1·49	1·64
Mill below Laughtendale ..	?–0·99	1·61	1·64
,, ,, ,, ...	,, ,,	1·59	
Spencer	1·05–1·32	1·57	1·67
,, ...	,, ,,	1·59	
Brooklyn	1·82–1·85	1·92	1·71–1·89
,, ...	,, ,,	1·87	

At the time of the investigation the water at Wiseman's Ferry had a salinity giving △ 0·38 at low tide. Although a more extensive search would be required for complete certainty, this seemed to be the freshest water in which the crabs were living, despite the fact that laboratory experiments indicated that they could live healthily, at least for some time, in more dilute water. It may be that this salinity (△ 0·38) is approximately the lowest for which they can live indefinitely, or it is more likely that in times of flood the salinity drops and then reaches the minimum for healthy life. A third and very important factor confining them to this region would be the unsuitability of the banks. No other flats where they are found in such abundance were observed for some distance higher up the river than Wiseman's Ferry. No *Heloecius* at all were found in the freshwater parts of the river, so it would seem that the death of this crab, when taken from sea-water and immersed in fresh water, is due not merely to inability to survive the sudden change, but to its being fundamentally impossible for this species to live in water devoid of salt. Thus another experimental conclusion is confirmed.

No other crabs have been examined in such detail as *Heloecius cordiformis*, but with several other species a small number of experiments have been made—sufficient to indicate that, in general, when in dilute solution, the relations between internal and external osmotic pressure are similar to those for *Heloecius*.

Several experiments parallel to those with *Heloecius cordiformis* were carried out for *Sesarma erythrodactyla*. Sometimes the two were examined at the same time and under exactly the same conditions. As in *Heloecius*, the blood of *Sesarma* became more dilute with addition of fresh water to the external medium. Again a slower rate of decrease in osmotic pressure for the blood made it hypertonic to the solution for any but slight dilutions. Thus in water, 80% of which was salt and whose △ was 2°, the blood of crabs after twenty days froze at –1·85° and –1·91°, while in 50% solution with the water freezing at –1·17° the blood froze at –1·64° when the crabs had been fourteen days in the medium.

These results also were confirmed by an examination of specimens found along the Hawkesbury River.

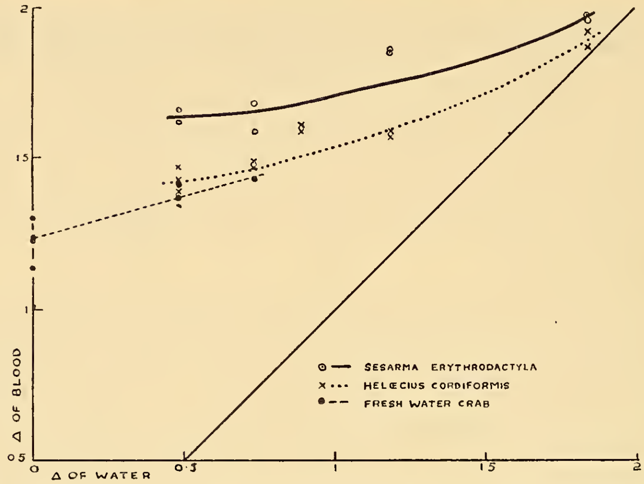
TABLE 9.
Sesarma erythrodactyla *on Hawkesbury River Banks.*

Place.	△ of Water.	△ of Blood.
Wiseman's Ferry	0·38–0·58	1·66
		1·62
Laughtendale	0·62–0·84	1·59
		1·68
Spencer	1·05–1·32	1·85
		1·86
Brooklyn	1·82–1·85	1·98
		1·96

That the internal concentration will vary according to species, even among animals of the same Order, is shown by the points on the graph (Fig. 4), which gives the values for the △ of the blood of freshwater crabs in the localities which have been under consideration, as well as representing graphically the Tables 8 and 9.

A species of the crab *Macrophthalmus* provided another example of marked hypertonicity of the blood over the external fluid. The freezing point of its blood was as low as –1·42° in a solution containing only 25% of sea-water.

Yet another crab, *Leptograpsus variegatus*, has been examined, and it displays the same hypertonicity of the blood if it is placed in dilute solutions of sea-water. This crab has a habitat entirely different from that of the preceding species, and



Text-fig. 4.—Comparison of internal and external media for three species of crab in dilute water on the Hawkesbury River. (The diagonal represents equality of △ for the two media.)

it is therefore of especial interest to find that the behaviour of its blood, resulting from external dilutions, is the same. It is found only in a rocky environment on the actual coast or near the mouths of rivers and harbours. It is sensitive to dilution, but can live for at least six days in a 50% solution of sea-water. A solution containing 10% of sea-water appeared to be about the border line between those salinities which were too low for the animal to survive and those in which it was able to live healthily (a specimen immersed in such a solution survived for two or three days). Values of △ have been obtained only for 50% solutions. They are summarized in Table 10.

TABLE 10.
Leptograpsus variegatus *Immersed in a 50% Solution of Sea-Water.*

Duration of Immersion.	△ of Water. (° C.)	△ of Blood. (° C.)
43 hours	1·07	1·79
43 hours	—	1·89
6 days	1·20	1·73

Thus the results for Australian species combine with many of those obtained by investigators elsewhere to show that a "law of poikilosmoticity" is by no means applicable to all marine invertebrates when the surrounding water is of lower salinity than ordinary sea-water. It is, however, of greater validity when the external concentration is increased above that of ocean sea-water.

Both Frédéricq and Duval found isotonicity in different species of crabs under conditions of increased salinity. The former found identity of internal and external freezing points for *Carcinus maenas* when immersed for three days in water of △ 3·11° and △ 3·84°. The latter obtained his result from *Platycarcinus* *pagurus*, *Palinurus vulgaris*, *Maja squinado* and *Carcinus maenas* immersed in various solutions which froze between –2° and –3°.

The crab *Heloecius cordiformis* was examined under conditions of increased external concentration. It can endure a large increase in the concentration of the external water, and will live for some time at least in double strength sea-water.

The previous notation for strength of solution will be used for setting out these results. Thus a 100% solution is one of normal concentration, 150% is a solution half of ordinary sea-water and half of double strength sea-water, and so on.

When *Heloecius* was first put into concentrated sea-water the specimens were left only for a day or two before the freezing point of the blood was determined, as this time was all that was necessary for the completion of the changes in dilute solutions. The results were very surprising in comparison with those of Duval, which showed isotonicity for *Carcinus* in 26 hours. In water of nearly double strength (△ 4·14°) the blood of *Heloecius* gave a △ equal only to 2·50°. Again, for a 150% solution of △ 3·14°, the △ for the blood was 2·18° on one occasion of immersion for 38 hours, and 2·29° on another when the experiment lasted 42 hours. A large number of experiments were subsequently carried out, and they all showed that the blood remains hypotonic to a large degree when the external medium is concentrated above the normal, and the time of immersion only one or two days.

It was now necessary to determine whether this hypotonicity was permanent, or whether it was due to a slower rate of change than under conditions of dilution. The latter alternative was found to be the true one.

Examination over a long period showed that the internal change is at first fast, but becomes gradually slower. Instead of ceasing altogether after a day or so (as is the case for dilutions) the change continues at a slow rate, until the salinities of the inner and outer media are almost equal (the difference being no larger than that for crabs in ordinary sea-water). The time necessary for the completion of the change was not constant, but varied from two to five weeks.

The rate of change can best be seen from the following tables and the graph (Fig. 5), which unite the results of typical experiments.

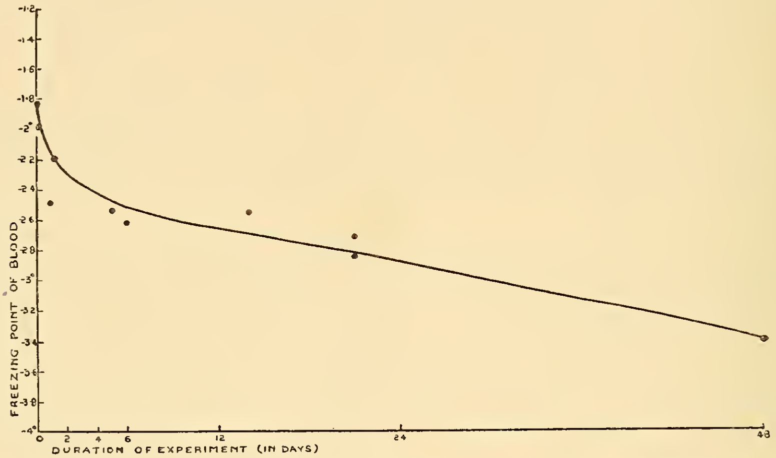
TABLE 11.
Heloecius cordiformis *Immersed in Solutions of* $\triangle = 3 \cdot 30 - 3 \cdot 40$.

Duration of Experiment.	△ of Blood. (° C.)	Difference between the Two Media. (° C.)
0	1·83	1·49
4 hours	1·98	1·34
21 hours	2·49	0·89
28 hours	2·19	1·21
5 days	2·54	0·78
6 days	2·62	0·70
14 days	2·56	0·82
21 days	2·72	0·68
21 days	2·85	0·55
48 days	3·41	*

* The external concentration would have been a little altered by evaporation and, as it was not obtained at the end of the experiment this difference must be omitted.

TABLE 12.
Heloecius cordiformis *Immersed in Various Solutions of Increased Concentration.*

Duration of Experiment.	△ of Solution. (° C.)	△ of Blood. (° C.)
6 days	2·37	1·96
7 days	2·64	2·18
28 days	3·28	2·92
29 days	3·17	3·15
36 days	3·24	3·10
36 days	2·42	2·28



Text-fig. 5.—*Heloecius cordiformis* immersed in solutions of △ 3·30-3·40.

Thus we find that *H. cordiformis* agrees with the marine invertebrates previously investigated in that, after an increase in the concentration of the surrounding water, its blood comes into the same relation with that water as it was originally with the ordinary sea-water. It differs considerably, however, from the crabs examined by Duval and Frédéricq in the length of the time taken to complete the internal change.

SUMMARY.

A number of species of Australian crabs from diverse habitats, such as a typical ocean coast, estuarine flats, and fresh waters, have been examined in order to determine the relationship of their body fluids to the external medium under natural and under experimental conditions.

Five species of crabs have been found to be homoiosmotic in diluted sea-water. This gives much additional support to the view that the condition may be regarded as general for the crustacea.

The number of species of marine crabs in which, under normal circumstances, there is a distinct anisotonicity between the body fluids and the external medium, has been increased. The difference is on the side of hypotonicity.

It is noticeable that, as in other cases, *Heloecius cordiformis* survives in highly diluted sea-water for a much longer time than in fresh water. The effect of the trace of salts is important.

The osmotic pressure of the blood varies amongst the individuals of any one species when taken from the same conditions and the same locality.

Although *Heloecius cordiformis* behaves like certain previously examined European crustacea in that its blood comes ultimately to a poikilosmotic condition when the crab is placed in water of increased concentration above normal ocean salinity, it differs from the known cases in taking about a month to reach this condition in highly concentrated solutions.

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